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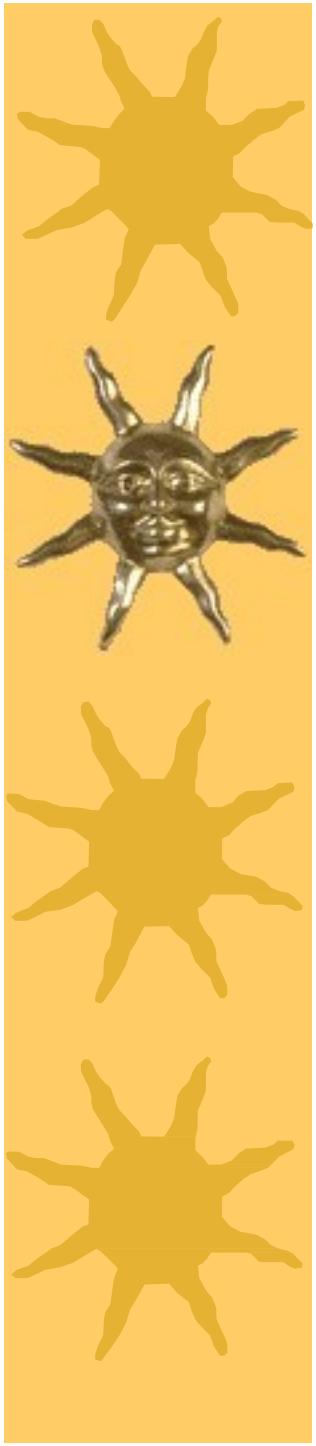
# FACULTY OF NURSING



# *OXIDATIVE STRESS,* APOPTOSIS & AGING

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# *Theories of aging*

←Theories of Aging:

←attempt to explain the phenomenon of aging as it occurs over the lifespan

- aging is viewed as a total process that begins at conception
- *senescence:* a change in the behavior of an organism with age leading to a decreased power of survival and adjustment

# *Theories of Aging: Types*

←Biologic

←Sociologic

←Psychologic

←Moral/Spiritual

# *Biologic Theories:*

- ←Concerned with answering basic questions  regarding the physiological processes that  occur in all living organisms as they  chronologically age

# *Foci of Biologic Theories*

←Explanations of:

- 1) deleterious effects leading to decreasing function of the organism
- 2) gradually occurring age-related changes that are progressive over time
- 3) intrinsic changes that can affect all member of a species because of chronologic age

←ALSO:

- all organs in any one organism do not age at the same rate

- any single organ does not necessarily age at the same rate in difference individuals of the same species

# *Biologic Theories: Divisions*

←*Stochastic:* Explain aging as events that occur randomly and accumulate over time

←*Nonstochastic:* View aging as certain predetermined, timed phenomena

←*Stochastic Theories*

←Error Theory

←Cross-Linkage Theory

←Wear & Tear Theory

Free Radical Theory

# *Error Theory*

- ←Originally proposed in 1963
- ←Basis: 1)errors can occur in the transcription in any step of the protein synthesis of DNA
  - 2) error causes the reproduction of an enzyme or protein that is not an exact copy
  - 3) As transcription errors to occur, the end product would not even resemble the original cell, thereby compromising its functional ability

# *Error, cont'd*

- More recently the theory has not been supported by research
  - not all aged cells contain altered or misspecified proteins
  - nor is aging automatically or necessarily accelerated if misspecified proteins or enzymes are introduced into a cell

# *Free Radical Theory*

- Free radicals are byproducts of metabolism--can increase as a result of environmental pollutants
- When they accumulate, they damage cell membrane, decreasing its efficiency
- The body produces antioxidants that scavenge the free radicals

# *Free Rads, cont'd*

- In animal studies, administration of antioxidants postpones the appearance of diseases such as cardiovascular disease and CA

- Free radicals are also implicated in the development of plaques associated with Alzheimer's

# *Cross-Linkage Theory*

←Some proteins in the body become cross-linked, thereby not allowing for normal metabolic activities

←Waste products accumulate

←Result: tissues do not function at optimal efficiency

# *Wear & Tear Theory*

←Proposed first in 1882

←Cells simply wear out over time because of continued use--rather like a machine

←Would seem to be refuted by the fact that exercise in OA's actually makes them MORE functional, not less

←*Nonstochastic Theories:*

←Programmed Theory

←Immunity Theory

# *Programmed (Hayflick Limit)* Theory

- Based on lab experiments on fetal fibroblastic cells and their reproductive capabilities in 1961
- Cells can only reproduce themselves a limited number of times.
- Life expectancies are seen as preprogrammed within a species-specific range

# *Immunity Theory*

←*Immunosenescence:* Age-related functional diminution of the immune system

←Lower rate of T-lymphocyte ("killer cells") proliferation in response to a stimulus

←& therefore a decrease in the body's defense against foreign pathogens

# *Immunity, cont'd*

- Change include a decrease in humoral immune response, often predisposing older adults to:
  - 1)decreased resistance to a tumor cell challenge and the development of cancer
  - 2) decreased ability to initiate the immune process and mobilize defenses in aggressively attaching pathogens
  - 3) increased susceptibility to auto-immune diseases

# *EMERGING THEORIES OF* AGING

←Neuroendocrine Control (Pacemaker) Theory

←Metabolic Theory/Caloric Restriction

←DNA-Related Research

# *Neuroendocrine Control*

- "…examines the interrelated role of the neurologic and endocrine systems over the life-span of an individual".

- there is a decline, or even cessation, in many of the components of the neuroendocrine system over the lifespan

# *Metobolic Theory of Aging* (Caloric Restriction)

←"…proposes that all organisms have a finite amount of metabolic lifetime and that organisms with a higher metabolic rate have a shorter lifespan".

←Rodent-based research has demonstrated that caloric restriction increases the lifespan and delays the onset of age-dependent diseases

# *DNA-Related Research*

←Major Developments:

- Mapping the human genome ("…there may be as many as 200 genes responsible for contolling aging in humans")

- Discovery of telomeres

# *SOCIOLOGIC THEORIES OF* AGING

- ←Disengagement Theory
- ←Activity/Developmental Task Theory
- ←Continuity Theory
- ←Age Stratification Theory
- ←Person-Environment Fit Theory

# *Person-Environment Fit Theory*

- ←Lawton, 1982
- ←Individuals have personal competencies that assist in dealing with the environment:
  - ego strength
  - level of motor skills
  - individual biologic health
  - cognitive & sensory-perceptual capacities

# *P-E Fit, cont'd*

- ←As a person ages, there may be changes in competencies & these changes alter the ability to interrelate with the environment

- ←Significant implications in a society that is characterized by constantly changing technology

# *PSYCHOLOGIC THEORIES* OF AGING

←Maslow's Hierarchy of Human Needs

←Jung's Theory of Individualism

←Erikson's Eight Stages of Life

←Peck's Expansion of Erikson's Theory

←Selective Optimization with Compensation

# *Oxidative stress (accumulation* of oxygen derived free radicals)

- Cell injury induced by free radicals ,particularly ROS,is an important mechanism of cell damage in many pathological conditions, such as chemical and radiation injury,ischemia reperfusion injury cellular aging and microbial killing by phagocytosis .
- Although O2 is lifeline of all cells and tissues ,its molecular form as ROS can be most devastating for cells .

# *What is free radicals -?*

- Free radical is a molecule or molecular fragment that contains one or more unpaired e- in its outermost orbit.
- Unpaired e- are highly reactive and attack and modify adjacent molecules such as inorganic and organic chemicals –proteins ,lipids ,carbohydrates,nucleic acids .
- Free radical is generally represented by a superscript dot,R*.
- Oxidation reactions ensure that molecular oxygen is completely reduced to water, the products of partial reaction of oxygen are highly reactive and create havoc in the living system, hence called ROS.

# *ROS*

- ROS are type of O2 derived free radical whose role in cell injury is well established .
- ROS are normally produced in cells during mitochondrial respiration and energy generation ,but they are degraded and removed by cellular defence systems.
- Increased production or decreased scavenging of ROS may lead to excess of these free radicals ,a condition called oxidative stress.
- Oxidative stress has been implicated in a wide variety of pathological process ,including cell injury ,cancer,aging and some degenerative diseases such as ALZHEIMER DISEASE.

*Ros are also produced in large amounts by activated leucocytes ,particularly neutrophils and macrophages*

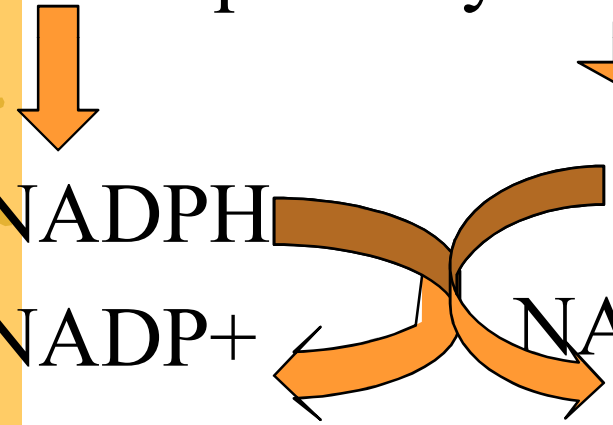
- Generation of free radicals :-
- They are constantly produced during the normal oxidation of food stuffs ,due to leak in ETC in mitochondria .
- About 1-4% O2 taken up in body is converted to free radicals .
- Mitochondria Are major sites for production of superoxide ions from the interaction between coenzyme Q and o2 in the ETC, hence a high content of SOD is needed

# 1.The reduction oxidation reaction:-

- That occur during normal metabolic processes ,as a part of normal respiration ,molecular o2 is reduced by the transfer of 4e- to H2 to generate two water molecules .
- This conversion is catalyzed by oxidative enzymes in the ER,cytosol mitochondria , peroxisomes and lysosomes .
- During this process small amount of partially reduced intermediates are produced ,
- Superoxide ⟶ H2O2 ⟶ OH*

# 2. NADPH OXIDASE

- NADPH oxidase in inflammatory cells produces superoxide anion by a process of respiratory burst during phagocytosis .
- HMP shunt pathway macrophages(respiratoryburst
- GPD




O2

NADPH oxidase

O2*- SOD H2O2 HCLo

bacteria killed

*In chronic granulomatous disease(CGD),the NADPH oxidase is absent in macrophages and neutrophils .*

- In this condition macrophages ingest bacretia normally but cannot destroy them ,hence recurrent pyogenic infection by staphylococci are common in CGD
- 3.Absorption of radiant energy,ionizing water can hydrolyze water into *OH and hydrogen free radicals .
- 4.enzymatic metabolism of exogenous chemicals or drugs can generate FR that are not ROS but have similar effects ,eg-CCL4 can generate *CCL3.

*5.Transition metals such as iron and copper donate or accept free e- during intracellular reaction and catalyze FR formation ,as in Fenton reaction-*

←Fe2+ +H2O2 ⟶ Fe3+ +*OH +OH-

←O2 +H2O2 —Fe→ O2+*OH+OH-(Heber weiss rea)

←6.NO- An imp chemical mediator generated by endothelial cells,macrophages,neurons and other cell types can act as FR and can also be converted to highly reactive peroxynitrite anion (ONOO-) as well as NO2 and NO3- .

# *Free radical scavenging systems*

- FR are inherently unstable and generally decay spontaneously .*O2- , for eg is unstable and decays spontaneously to O2 and H2O2 in presence of H2O
- Cells have developed multiple non enzymatic and enzymatic mechanisms to remove FR and thereby minimize injury.
- Fe and Cu catalyze the formation of ROS, under normal circumstances ,the reactivity of these metals are minimized by their binding to storage and transport proteins ,eg- Transferrin, Ferritin, lactoferin and ceruloplasmin, which prevent these metals from participating in reactions that generate ROS.

# system

- 1. CATALASE- present in peroxisomes ,decomposes H2O2.
- 2H2O2 catalase > O2+2H2O
- 2. SOD – found in many cell types and convert *O2 to H2O2
- 2*O2+2H ⟶ H2o2+O2,
- This group of enzymes include both maganese – SOD, localised in mitochondria and Cu-Zn SOD,found in cytosol.
- A defect in SOD gene is seen in patients with amyotrophic lateral scleroris.

*3.Glutathione peroxidase also protects against injury by catalyzing free radical breakdown*

$H_2O_2 + 2GSH \longrightarrow GSSG + 2H_2O$ or

$2{}^*OH + 2GSH \longrightarrow GSSG + 2H_2O$

←GSSG(glutathione homodimer)

←The intracellular ratio of oxidised glutathione (GSSG) to reduced glutathione (GSH) is the reflection of the oxidative state of the cell ,and an imp indicator of the cells ability to detoxify ROS.

4.Glutathione reductase –the GSSG $\xrightarrow{NADPH}$ GSH this NADPH is generated with the help of G6PD in HMP shunt pathway .

Therefore in GPD deficiency ,RBCs are liable to lysis ,especially when oxidizing agents are administered (drug induced hemolytic anemia).

## represent a wide variety of compound

s

- That occur in fruits ,vegetables , wine ,tea and chocolate ,
- They contain flavones, isoflavones , flavonols,catechins and phenolic acids ,they work as agents having antioxidants, antiapoptotic ,antiaging,anticarcinogenic, antiartherosclerotic effects
- They are protective against cardiovascular diseases .

# *Pathological effects of free radicals*

- 1.Lipid peroxidation of membranes- in presence of O2 free radicals may cause peroxidation of lipids within plasma and organellar membranes
- Oxidative damage is initiated when the double bond in unsaturated fatty acids of membrane lipids are attacked by O2 derived free radicals .
- 2.Protein modification-free radicals promote oxidation of aa side chains, formation of covalent protein –protein crosslinks (.eg.disulfide bonds ) and oxidation of protein backbone .
- Oxidative modifications of proteins may damage the active sites of enzymes –disrupt thre conformation of structural proteins and loss of function and fragmentation of proteins ,raising havoc throughout the cell.

# 3. DNA damage (lesions of DNA)

- FR are capable of causing single and double stranded breaks in DNA ,cross linking of DNA strands .
- Clinical ~~significance~~ of ~~FR~~
- 1 chronic inflammation(rheumatoid arthritis,chronic glomerulo nephritis,chronic ulcerative colitis.)
- 2 Acute inflammation
- 3 respiratory disease
- 4 disease of eye (retrolental fibroplasia in premature on exposure to 100% O2, causes .
- 5 reperfusion injury-during ischemia activity of xanthine oxidase increases ,when reperfused cause HYPOXANTHINE to xanthine and superoxide .
- 6 Arherosclerosis and MI
-
-

# *Role of antioxidants*

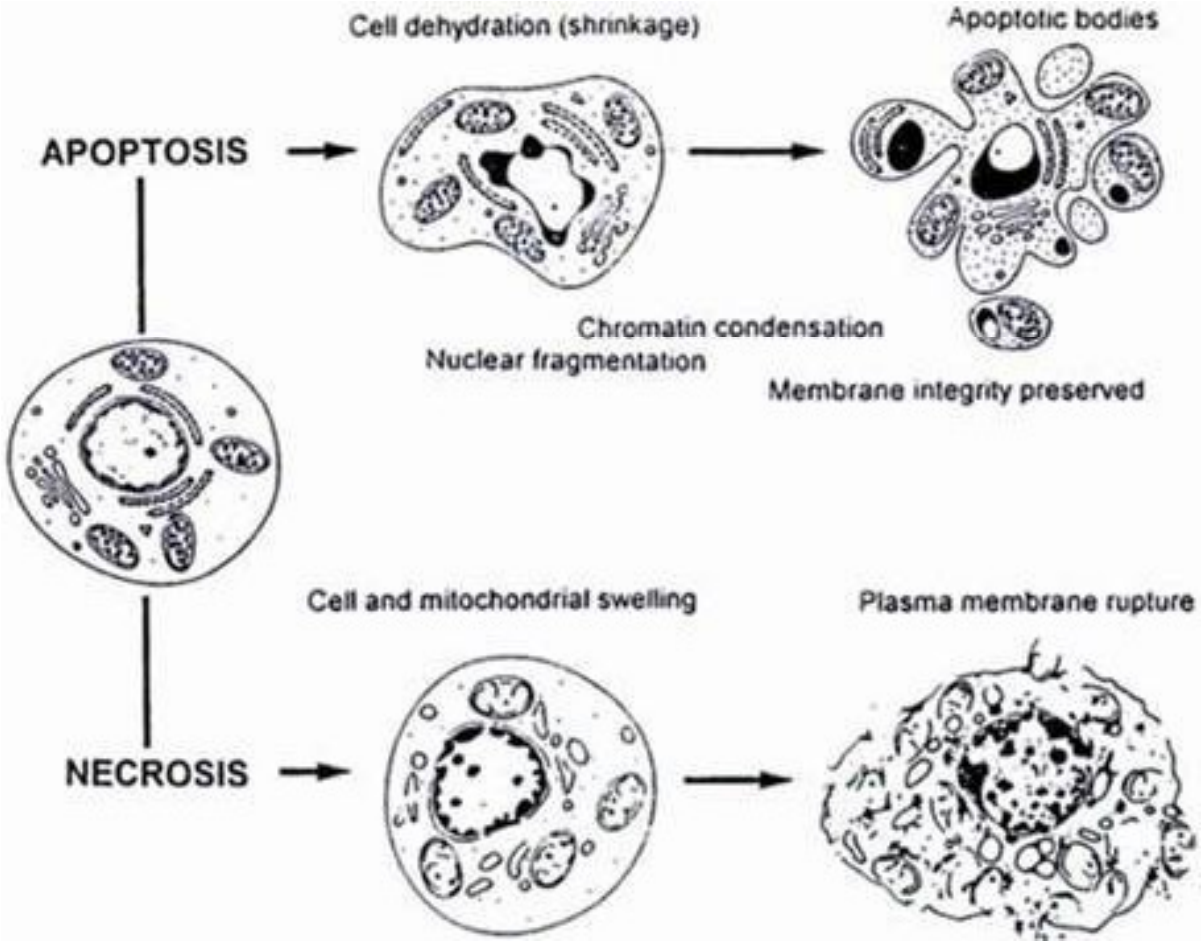
- Preventive antioxidants:- inhibit the initial production of free radicals ,they are catalase, glutathione peroxidase and EDTA.
- Chain breaking antioxidants :- inhibits propagative phase . They include superoxide dismutase ,uric acid and vitE
- VitE - most effective naturally occurring chain breaking antioxidant in tissues .
- VitE is lipid phase antioxidant
- VitC is aqueous phase antioxidant
- Ceruloplasmin can act as antioxidant in ECF.

# *APOPTOSIS*

- Apoptosis is a pathway of cell death that is induced by a tightly regulated sucide program in which cell destined to die activate intrinsic enzymes that degrade the cells own nuclear DNA and nuclear and cytoplasmic proteins.
- Apoptotic cells breakup into fragments called apoptotic bodies which contains the portion of cytoplasm and nucleus .
- The plama membrane of apoptotic cells and bodies remains intact ,but structure altered in such a way that it becomes *tasty* target for phagocytes.
- Dead cell and its fragments are rapidly devoured before the contents have leaked out, therefore cell death by this pathway does not elicit inflamatory reaction in the host.
- It was quickly appreciated that apoptosis was unique mechanism of cell death ,distinct from necrosis.

# *Death by Injury vs. Death by Suicide*

## (Necrosis vs. Apoptosis)



# *Necrosis vs. Apoptosis*

| Necrosis | Apoptosis |
| --- | --- |
| • Cellular swelling | ← Cellular condensation |
| • Membranes are broken | ← Membranes remain intact |
| • ATP is depleted | ← Requires ATP |
| • Cell lyses, eliciting an inflammatory reaction | ← Cell is phagocytosed, no tissue reaction |
| • DNA fragmentation is random, or smeared | ← Ladder-like DNA fragmentation |
| • In vivo, whole areas of the tissue are affected | ← In vivo, individual cells appear affected |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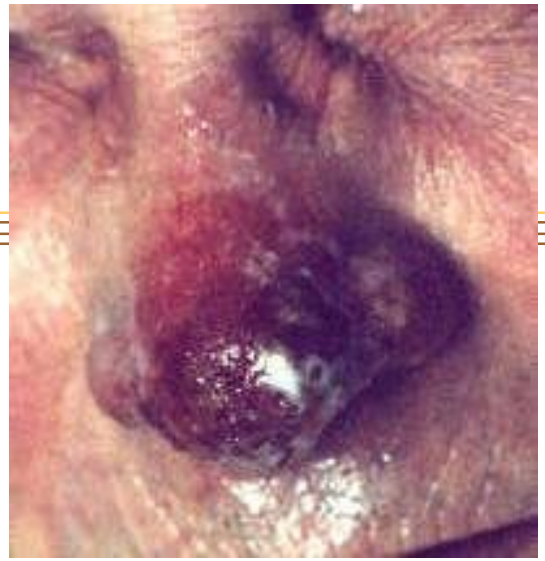
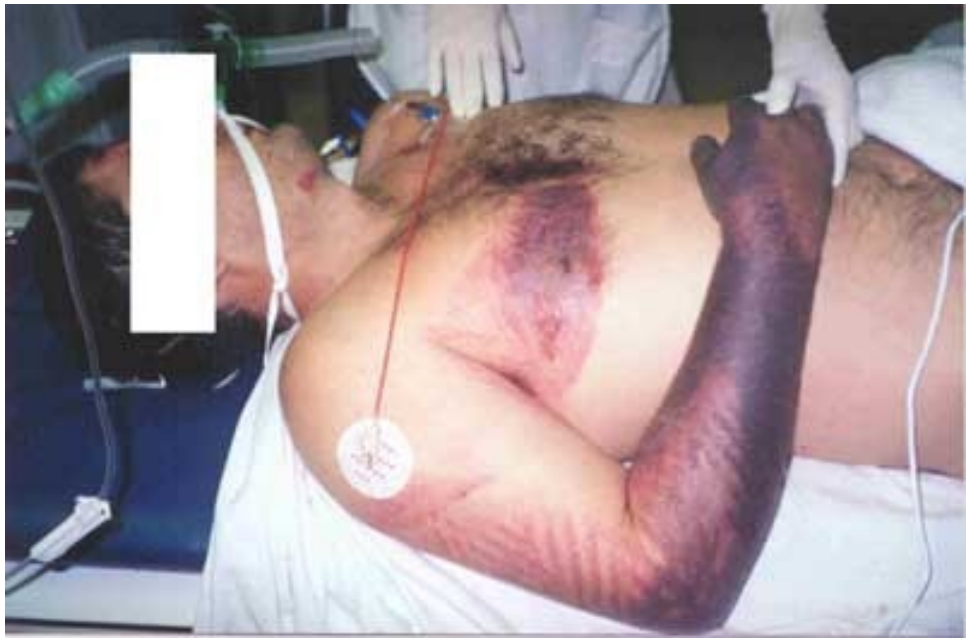




# Necrosis

*Trauma (toxic chemicals, mechanical injury, heat, hypoxia)*

*Loss of ability to regulate internal environment*

*Ca2+ influx accompanied by swelling*

*Alteration of protein*

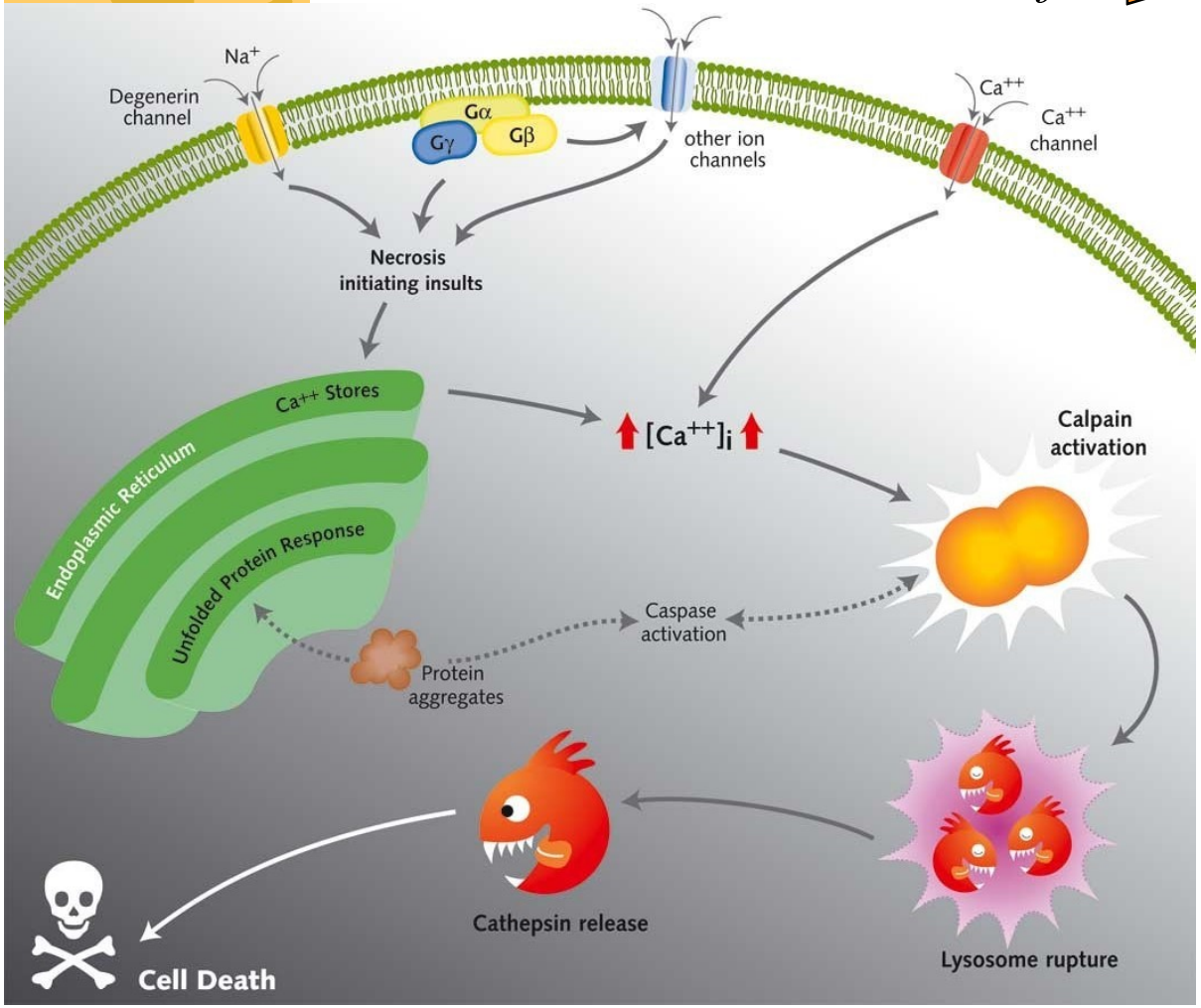
*calpain*

*cathepsin*

*caspase*

*Production of toxic*

*(activation of*



# *MECHANISMS OF APOPTOSIS*

- Activation of cysteine proteases in the cell called *CASPASES triggers apoptosis.*
- Caspases are stimulated by external and internal stimuli.
- Internal stimuli: mitochondria release cytochrome and a protein called smac that causes activation of the caspases 9,which induces apoptosis.
- EXTERNAL STIMULI: are various ligands that bind with cell surface to activate apoptosis .one such factor is tumor necrosis factor that activates the enzyme CASPASE 8.
- Caspase activation promotes DNA FRAGMENTATION AND CHROMATIN CONDENSATION.

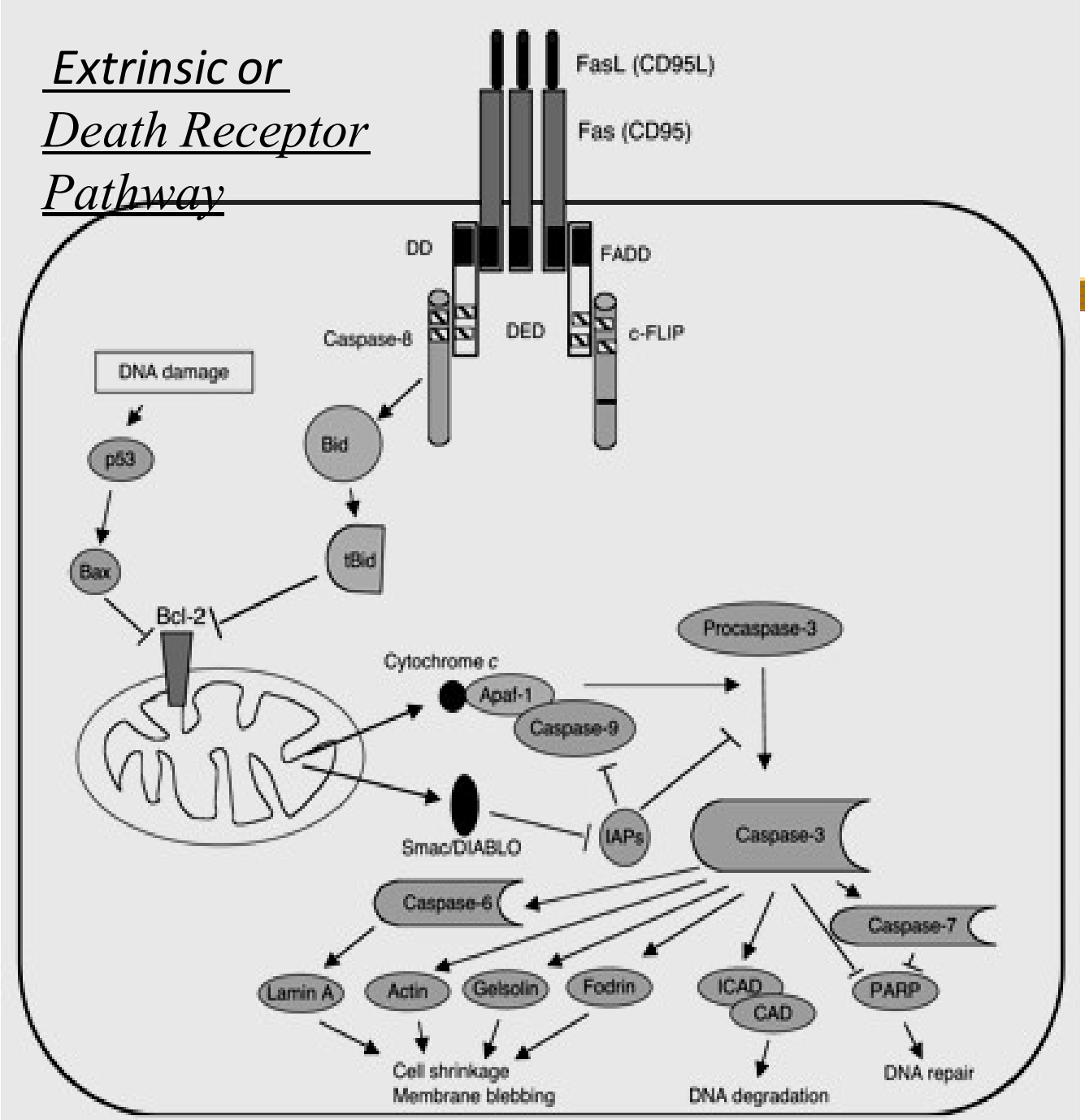
**Apoptosis results from activation of enzymes called caspases(cysteine proteases that cleave proteins after aspartic residues)**

- PRESENCE OF CLEAVED ACTIVE CASPSES IS A MARKER FOR CELL UNDERGOING APOPTOSIS
- PROCESS OF APOPTOSIS IS DIVIDED INTO INITIATION PHASE DURING WHICH SOME CASPASES BECOME CATALYTICALLY ACTIVE
- ,
- EXECUTION PHASE DURING WHICH OTHER CASPASES DEPENDS TOTALLY ON A FINELY TUNED BALANCE BETWEEN PROAPOPTOTIC AND ANTIAPOPTOTIC PROTEINS

# *Two distinct pathways –*

- A.EXTRINSIC pathway of apoptosis :- this pathway is initiated by engagement of plasma membrane death receptors on a variety of cells
- Death receptors are members of TNF receptor family that contain a cytoplasmic domain involved in protein-protein interactions that is called death domain becoz its is essential for delivering apoptotic signals .
- The best known death receptors are the type 1TNF receptors and a related protein called FAS.

# *Extrinsic or Death Receptor Pathway*




- *Binding of Fas by FasL induces recruitment of FADD to the cytoplasmic tail of Fas*
- *The opposite end of FADD contains a death effector domain (hatched boxes); recruitment of either procaspase-8 or c-FLIP*
- *Caspase-8 can cleave Bid*
- *truncated Bid (tBid) can inactivate Bcl-2 in the mitochondrial membrane.*
- *This allows the escape of cytochrome c, which clusters with Apaf-1 and caspase-9 in the presence of dATP to activate caspase-9.*
- *Smac/DIABLO is also released from the mitochondria and inactivates inhibitors of apoptosis (IAPs).*
- *breakdown of several cytoskeletal proteins and degradation of the inhibitor of caspase-activated DNase (ICAD).*

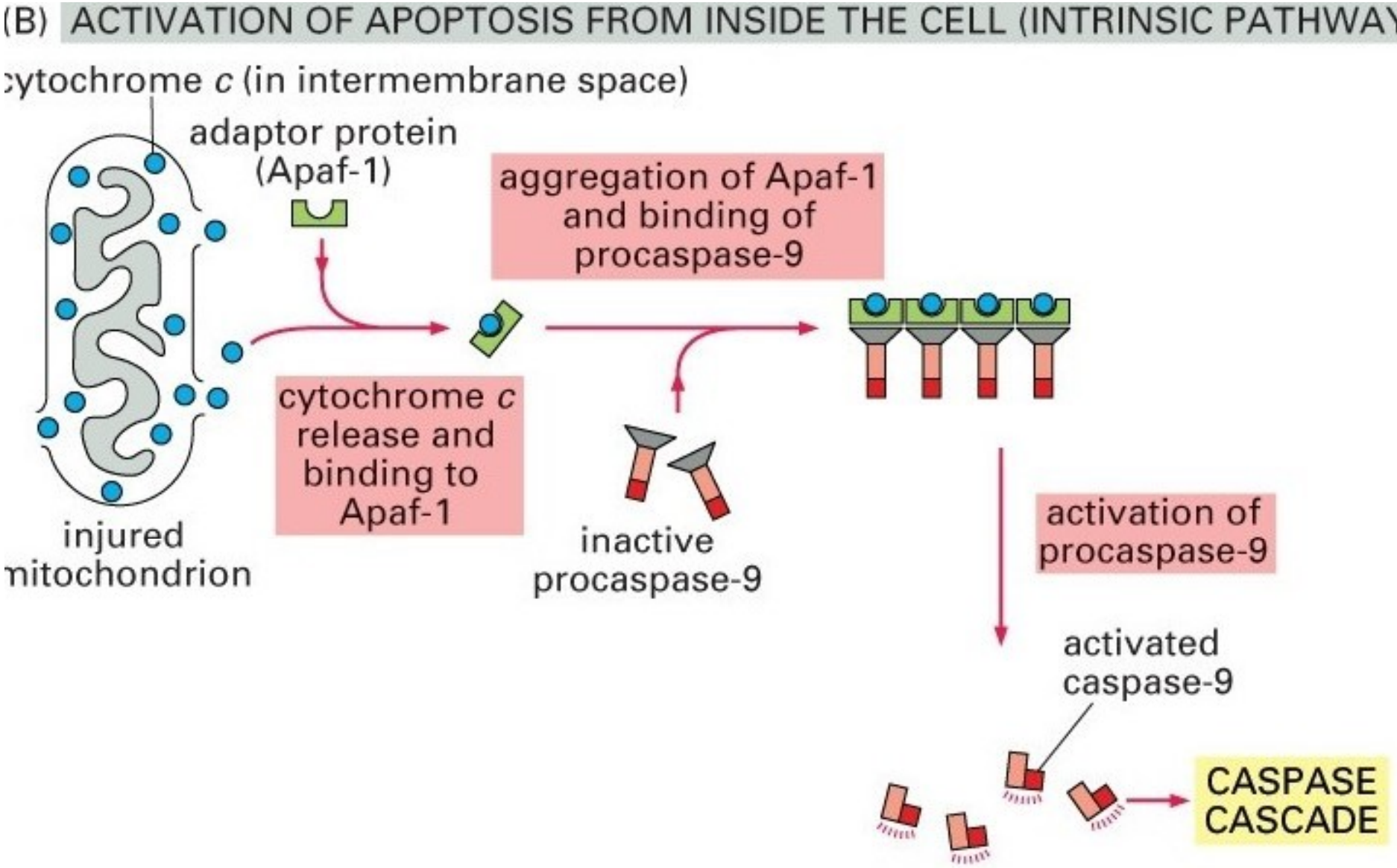



Figure 17–39 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

caspase-3 (*right panel*, immunofluorescence stain with an antibody specific for the active form of caspase-3, revealed as red color). (By courtesy of R. Hamm BV: Definition and incidence of apoptosis: a historical perspective. In Tomei LD, Cope FO (eds): Apoptosis: The Molecular Basis of Cell Death. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1991, pp 5-29; **C,** Courtesy Dr. Zheng Dong, Medical College of Georgia, Augusta, Ga.)

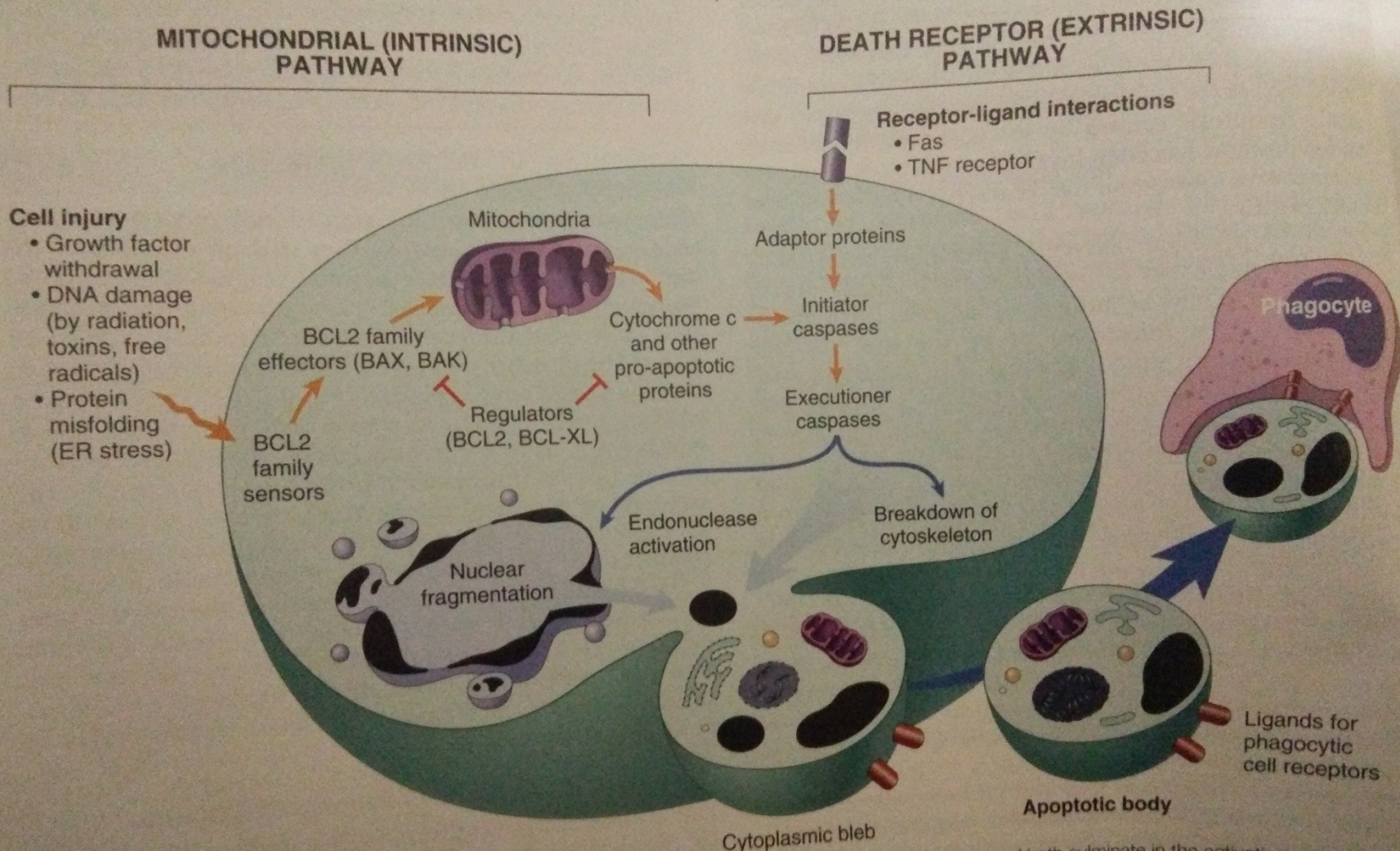
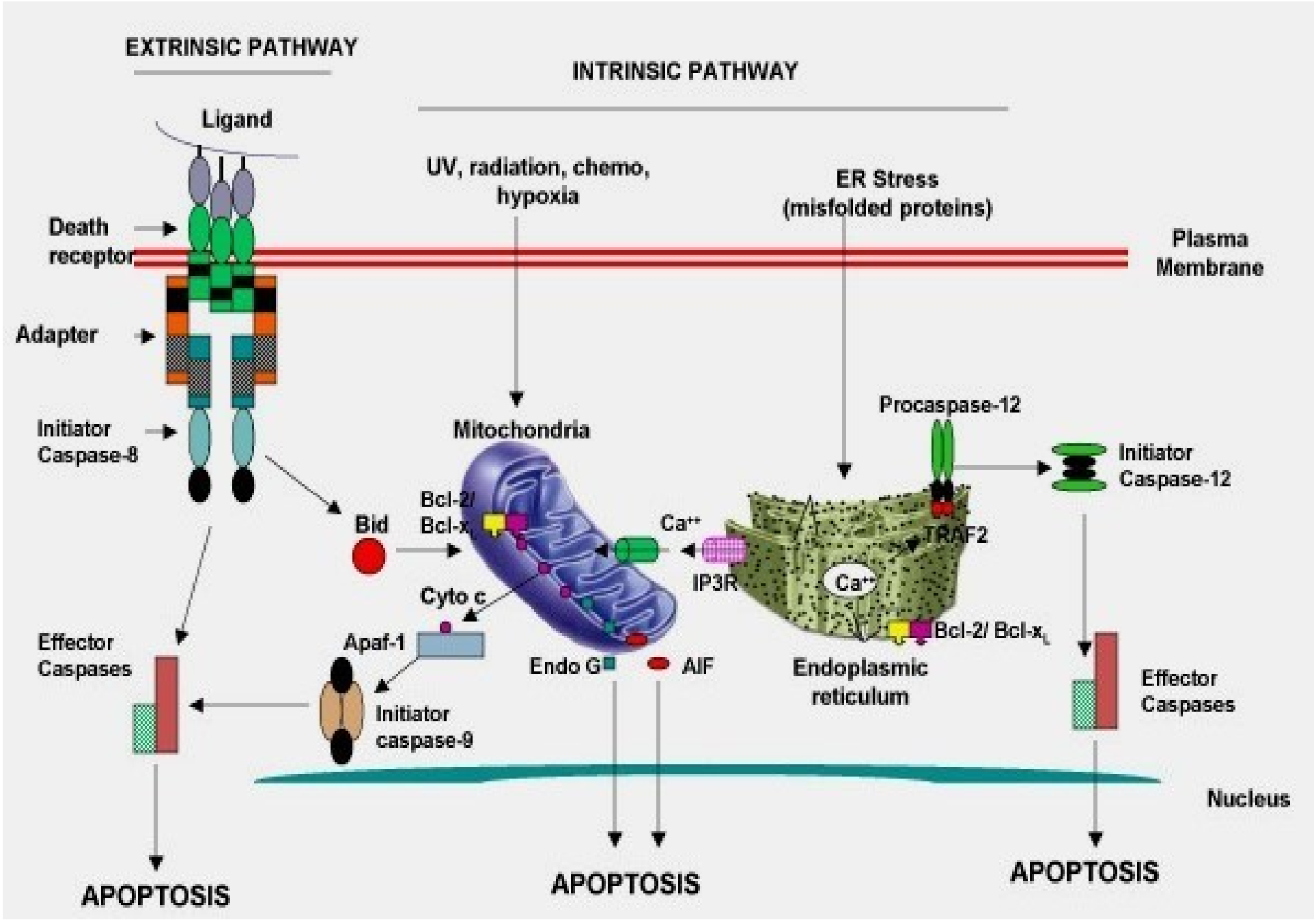



**Figure 2-23** Mechanisms of apoptosis. The two pathways of apoptosis differ in their induction and regulation, and both culminate in the activation of caspases. In the mitochondrial pathway, proteins of the BCL2 family, which regulate mitochondrial permeability, become imbalanced and leakage of various substances from mitochondria leads to caspase activation. In death receptor pathway, signals from plasma membrane receptors lead to the assembly of adaptor proteins into a "death-including signaling complex," which activates caspases, and the end result is the same.

*In the mitochondrial pathway ,proteins of the BCL2 family ,which regulate mitochondrial permiability become imbalanced and leakage of various substance,* from the ~~mitochondria leads t~~o caspase activation .

In death receptor pathway ,signals from the plasma membrane receptors lead to the assembly of adaptor proteins into a Death –Inducing signaling complex, which activates caspases .and the result is the same .


EXTRINSIC PATHWAY
INTRINSIC PATHWAY
Ligand
UV, radiation, chemo, hypoxia
ER Stress (misfolded proteins)
Death receptor
Plasma Membrane
Adapter
Initiator Caspase-8
Mitochondria
Procaspase-12
Initiator Caspase-12
Bid
Bcl-2/ Bcl-x
Ca++
TRAF2
IP3R
Ca++
Cyto c
Bcl-2/ Bcl-xL
Effector Caspases
Apaf-1
Endo G
AIF
Endoplasmic reticulum
Effector Caspases
Initiator caspase-9
Nucleus
APOPTOSIS
APOPTOSIS
APOPTOSIS

*Apoptosis is involved in*

- *Cancer ( via HPV, Epstein bar virus; melanoma)*
- *regulation of the immune system,*
- *AIDS,*
- *organ transplants*

**Table 3** Correlation between HPV-16 E6 infection and p53 mutation

| | HPV-16 | |
|---|---|---|
| p53 | Positive | Negative |
| Positive | 14 | 7 |
| Negative | 12 | 17 |

$P > 0.05$; $\chi^2$ test.

***Melanoma*** *(the most dangerous type of skin cancer) cells avoid apoptosis by inhibiting the expression of the gene encoding* ***Apaf-1****.*

# *FUTURE PERSPECTIVES*

- The biological roles of newly identified death receptors and ligands need to be studied

- Need to know whether defects in these ligands and receptors contribute to disease

# *THE END!*

←Thanks for hanging in there!